

Branched-chain amino acid aminotransferase along the rabbit and rat nephron

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Branched-chain amino acid aminotransferase along the rabbit and rat nephron. The activity of branched-chain amino acid aminotransferase (EC 2.6.1.42) is reported for four or five different segments of the rat and rabbit nephron as well as for patches from the papilla. In the rat the levels ranged 40-fold, from a high in the thick ascending limb of Henle to a low in the proximal convoluted tubule. The peak activity is far above that reported for most other parts of the body. Maximum activity was located also in the thick ascending limb in the rabbit, but the level was only one-third as high as in the rat. It is postulated that ammonia liberated by this amino transferase, in cooperation with glutamate dehydrogenase, could diffuse readily into the adjacent proximal straight tubule where all of the renal glutamine synthase and the highest level of alanine aminotransferase are located. Thus alanine and glutamine could be produced when the ammonia was not needed to neutralize excess acidity.

Amino-transférase d'acides aminés à chaîne branchée le long du néphron de lapin et de rat. L'activité de l'amino-transférase d'acides aminés à chaîne branchée (EC 2.6.1.42) est rapportée pour quatre ou cinq segments différents de néphron de rat et de lapin, ainsi que pour des fragments de papilles. Chez le rat, les niveaux variaient de 40 fois, d'une valeur haute dans la branche ascendante large de Henlé à une valeur basse dans le tubule contourné proximal. L'activité maximale est bien supérieure à celle rapportée pour la plupart des autres régions de l'organisme. L'activité maximale était située également dans la branche ascendante large chez le lapin, mais son niveau n'était que le tiers de celle du rat. Il est postulé que l'ammoniaque libéré par cette amino-transférase en association avec la glutamate deshydrogénase, pourrait diffuser facilement dans le tubule proximal droit adjacent où la totalité de la glutamine synthétase rénale et la plus grande quantité de l'alanine amino-transférase sont localisées. Ainsi, de l'alanine et de la glutamine pourraient être produits quand de l'ammoniaque n'est pas nécessaire pour neutraliser l'excès d'acidité.

The initiation of branched-chain amino acid metabolism occurs mainly in extrahepatic tissues, unlike that of most amino acids which are metabolized in the liver. The kidney has an especially high level of branched-chain amino acid aminotransferase (BCAAT, EC 2.6.1.42), the enzyme that initiates the catabolism of these amino acids [1–4]. Skeletal muscle [2] and adipose tissue [5], although lower than kidney in this enzyme, probably metabolize the bulk of these amino acids because of their larger mass. The kidney, however, carries out three major functions: gluconeogenesis, maintenance of acid base balance,

and reabsorption or excretion of amino acids, which are related to three prominent signs of maple syrup urinary disease [6] and other disorders due to genetic defects in the catabolism of branched-chain amino acids. These signs are hypoglycemia, acidemia, and elevated serum level of branched-chain amino acids and keto acids.

Interest in the metabolism of these amino acids and their keto acid analogues has been stimulated recently by observations that leucine accelerates the synthesis of rat muscle protein when muscle wasting occurs (starvation) and that α -keto isocaproate will serve in its place [7]. These studies raised the possibility of treating patients with muscle-wasting diseases with these acids. For example, patients with chronic renal failure and low protein intake can be maintained in nitrogen balance by supplementation with keto analogues of branched-chain amino acids [8]. Presumably, this is accomplished by their conversion via the transferase reaction into branched-chain amino acids [9, 10].

It seemed likely that a specialized enzyme, such as branched-chain amino acid transferase, might be distributed unevenly along a structure as heterogeneous as the nephron. Therefore, a suitable method was developed [11] for assay of the necessarily small samples involved, and the levels of this enzyme were measured in the various segments of the nephron of an omnivorous and an herbivorous animal (rat and rabbit) to find where these essential amino acids might be best utilized.

Methods

Kidney preparation. Animals were adult male white New Zealand rabbits weighing 1.5 to 3 kg and male Sprague-Dawley rats, 300 to 350 g. Rabbit kidneys were removed under nembutal anesthesia after clamping the renal vessels, cut sagittally into 3 slices (2 to 3 min), and frozen in freon-12 brought to its freezing point (-160°C) with liquid N_2 . Rat kidneys were obtained under light ether anesthesia and frozen whole within 2 sec, also in freon-12. Tissues were stored at -80°C until prepared for analysis. Frozen sections ($16\ \mu\text{m}$) were prepared at -20°C , freeze-dried at -35°C , and stored under vacuum at -25°C until used.

Nephron segments. The technique for isolation and weighing small identified samples of tissue from freeze-dried microtome sections has been described in detail [12, 13]. Each sample consisted of either a nearly central slice of a glomerulus, a 75 to $150\ \mu\text{m}$ length of proximal or distal tubule, or a patch from the papilla. Samples (10 to 40 ng dry wt) were weighed on a quartz

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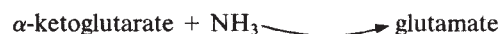
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fiber balance to $\pm 2\%$ [13]. Various parts of the nephron were identified by characteristic location, color, size, and texture [12, 14].

Enzyme assay. The study was made possible by the development of a new method [8] based on measuring the activity in the reverse of the usual direction:



followed by enzymatic measurement of the α -ketoglutarate:



The advantage of measuring the reaction in the reverse direction is that the product determined (α -ketoglutarate) is present at much lower levels in the kidney than is glutamate (the other choice). Therefore, the tissue blanks can be kept with manageable limits (10 to 30% of the transaminase contribution). The first four steps of the assay were carried out under oil (the oil-well technique [13]) to permit the use of very small volumes.

The samples from the nephron segments were introduced into 0.2 μl of the specific reagent which included α -ketoisocaproate, glutamate, and pyridoxal phosphate plus glycerol to stabilize the enzyme. After 60 min at 20°C, the reaction was stopped with an equal volume of dilute HCl, followed by a heat step to kill tissue enzymes. The α -ketoglutarate was then reduced to glutamate as shown above, and excess NADH was destroyed with HCl. The volume at this point was 1.6 μl . The NAD^+ , which had been produced in stoichiometric yield, then was amplified about 10,000-fold by enzymatic cycling [15].

Under the analytical conditions, the K_m for α -ketoisocaproate is very low (7 μM) and the level used (120 μM) is nearly saturating (95%). However, the K_m for glutamate is 15 mM and therefore the level used (10 mM) gives only 40% of the V_{\max} . Using higher glutamate levels made it more difficult to achieve stoichiometry at the second enzyme step. The precision of measurement was unaffected by the use of non-saturating glutamate levels, since only a minute fraction of this substrate was consumed. Linearity with time of reaction was excellent. This, however, was only true if glycerol was included in the reagent (at a level of 20%).

Results

BCAAT distribution in sequential kidney patches. In an orientation experiment, a single 16 μm freeze-dried cross section of rabbit kidney was divided into sequential patches from capsule to papilla for assay (Fig. 1). Each patch measured about 400 \times 700 μm . The highest activity was observed just below the border between the inner and outer stripe of the outer medulla, with levels falling off in a gradient in both directions to 25% of the peak at the outer cortex and 8% of the peak in the deep papilla. Tissue blanks (which have been deducted) are shown also in Figure 1.

These results suggested that high levels of BCAAT would be found in structures other than the proximal tubules.

Distribution of the enzymes along the nephron. Figure 2 depicts the distribution of BCAAT within both rat and rabbit nephron. Peak activity occurred in the distal straight tubule (thick ascending limb of Henle) in both species, but the absolute

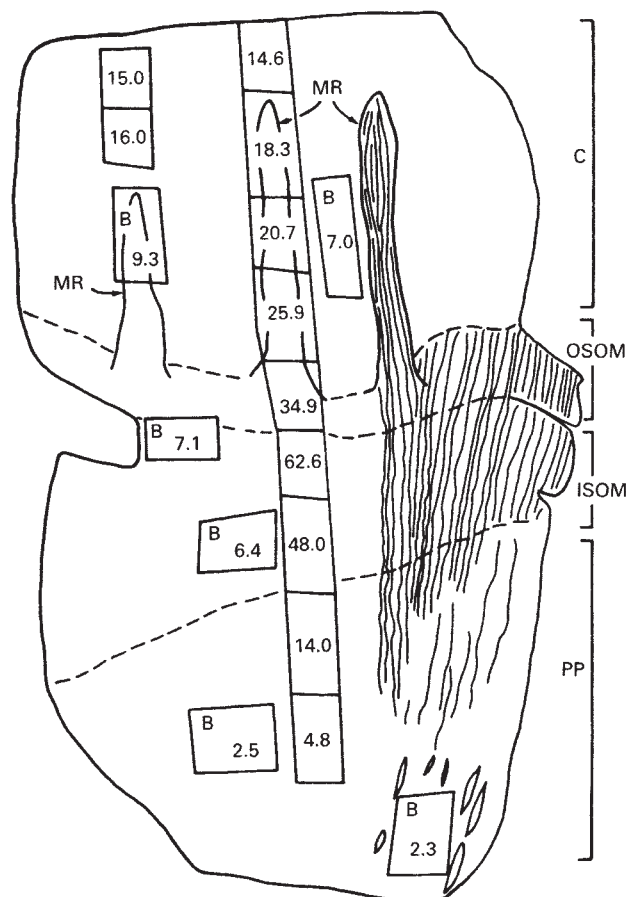


Fig. 1. Map of BCAAT in sequential samples from a 16 μm freeze-dried rabbit kidney cross section. Samples averaged about 1 μg dry wt and had an area of about 0.25 mm^2 . Activities are recorded as mmole/kg(dry)/hr at 20°C and have been corrected for tissue blanks measured on nearby samples labeled B. Abbreviations are: MR, medullary ray; C, cortex; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; PP, papilla.

level was 3 times greater in rat than in rabbit. The levels elsewhere were much lower, particularly in the rat, where the activity in the proximal convoluted tubule and glomerulus fell to about 3% of the peak. The low levels in the proximal convoluted segment are especially significant since this is where the most active amino acid reabsorption takes place [16].

BCAAT is present in mitochondria as well as the cytosol (2, 17–19). Moreover, peak levels of most mitochondrial enzymes are found also in the distal tubule. However, the differences between proximal and distal tubule concentrations are much less than seen for the BCAAT. This is illustrated by the distribution of fumarase, a typical mitochondrial enzyme, plotted on the same graph as BCAAT (Fig. 2). Fumarase, citrate synthase, and β -hydroxyacyl CoA dehydrogenase are all distributed similarly in the rat nephron [20].

Data are available [21] for the distribution along the rat nephron of two other transaminases: aspartate aminotransferase and alanine aminotransferase (Fig. 3). Each of the three enzymes has a distinctive pattern. The aspartate enzyme peaks in the same region as BCAAT, but is relatively much higher in glomerulus, proximal convoluted, and early proximal straight

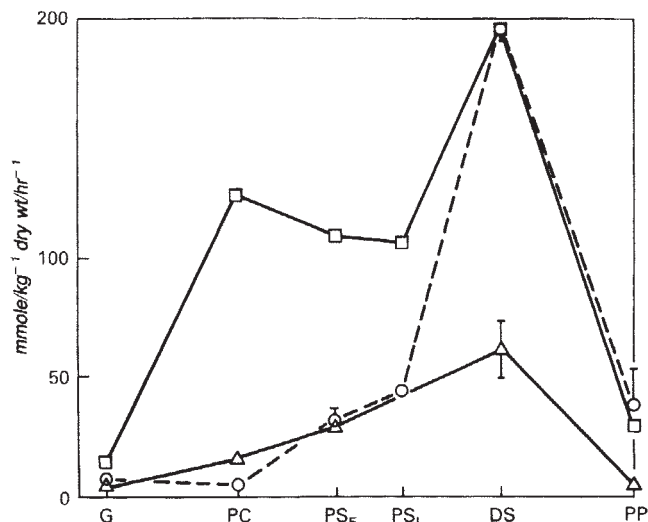


Fig. 2. Distribution of BCAAT in segments of rat and rabbit nephron. Activities are mmole/kg dry wt/hr \pm SEM for four rats and three rabbits except that PS_L is the average of only two animals. For each animal, four to eight samples of each segment were analyzed. Abbreviations are: G, glomerulus; PC, proximal convoluted tubule; PS_E and PS_L, proximal straight tubule from medullary ray and from the outer stripe of outer medulla, respectively; DS, distal straight tubule (thick ascending limb of Henle) from the inner stripe of outer medulla; PP, papilla area. For comparison, the distribution of a strictly mitochondrial enzyme, fumarase, from a previous paper [20], is shown also. Symbols are: \square , rat fumarase/225; \circ , rat BCAAT; \triangle , rabbit BCAAT.

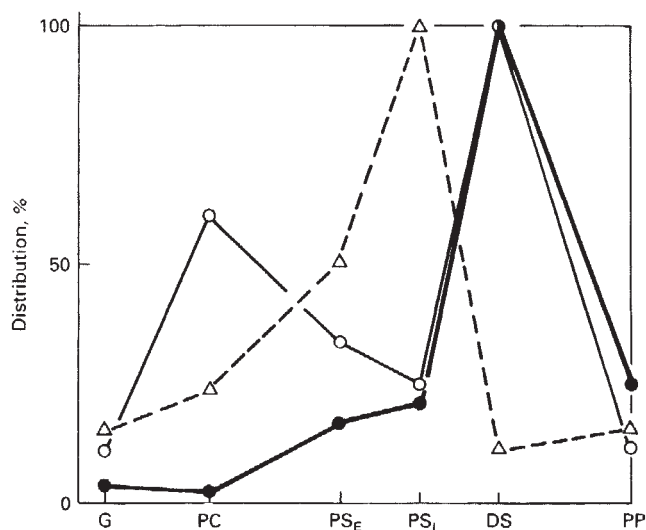


Fig. 3. Comparative distribution patterns of three amino acid transferases within the rat nephron. Alanine amino acid transferase (ALAAT) and aspartate amino acid transferase (ASPAT) are adapted from data of Chan et al [17]. Peak activities (mmole/kg dry wt/hr, all measured at 20°C) were BCAAT, 196; ALAAT, 173; ASPAT, 17,000. The BCAAT activity was measured with a glutamate concentration only two-thirds of the K_m . Therefore, the calculated V_{max} would be closer to 500 mmole/kg/hr. Abbreviations are the same as Fig. 2. Symbols are: \circ — \circ , ASPAT; \triangle — \triangle , ALAAT; \bullet — \bullet , BCAAT.

segments. Alanine aminotransferase peaks in the late proximal straight tubule and the ratio to BCAAT varies almost 100-fold from a high in the proximal convoluted tubule to a low in the

distal straight tubule. Note that peak absolute activities of BCAAT and alanine aminotransferase are similar, whereas aspartate aminotransferase activity is higher by two orders of magnitude.

As indicated, the BCAAT activities were measured in the reverse direction, that is, in the direction of branched-chain amino acid formation. We do not have accurate data for the ratio of maximum velocities in the two directions. However, the forward rate may be somewhat faster. The equilibrium constant for the reaction with leucine is 1.75 in favor of deamination [18]. Ichihara and Koyama [2] with pig heart BCAAT found a 2:1 velocity ratio between forward and reverse velocities. The substrate levels were all 6.7 mM. Consequently, the ratio between the two V_{max} would probably be somewhat lower because the K_m for glutamate is probably much higher than 6.7 (15 mM in rabbit and rat kidney [11]).

Discussion

The data presented demonstrate that branched-chain amino acid aminotransferase is distributed very unevenly along the nephron, and that in the segment with peak concentration it is probably higher by a wide margin than anywhere else in the body (exceptions may be stomach and pancreas [3]). Nevertheless, this enzyme is almost absent from the proximal convoluted tubule, the segment most active in reabsorptive capacity for amino acids [16].

To understand the significance of BCAAT for kidney it may be useful to focus on the site where it is highest, the ascending thick limb of Henle. Here the activity is 40 times that of alanine aminotransferase, the second most active transaminase in most cells, and the calculated V_{max} at 38° is more than 6 mmoles/kg wet wt/min. In skeletal muscle there is good evidence that ammonia removed from branched-chain amino acids is transferred via glutamate to form glutamine and alanine. At first glance this seemed unlikely in the case of the kidney because of the relatively low level of alanine aminotransferase in the thick ascending limb (Fig. 3), 2 to 5% of that in muscle, and the complete absence of glutamine synthase [22]. However, all of the renal glutamine synthase is concentrated in the proximal straight tubule [22], which is closely apposed to the ascending limb, and here also is located the highest concentration of alanine aminotransferase (Fig. 3). It is, therefore, not hard to imagine that the NH_3 might be shunted back into the proximal straight tubule to form glutamine. Good et al [23] have recently demonstrated that ammonia can in fact be transported from the lumen of the thick ascending limb of Henle against a gradient into the surrounding bath. Alternatively, when necessary, the NH_3 could be used to neutralize excess acidity, and for this purpose would be generated in a very favorable location.

An additional function for the high BCAAT in the thick ascending limb might be to rescue branched-chain ketoacids released from muscle and convert them back into the respective (essential) amino acids. Mitch and Chan [9] found that when the isolated rat kidney was perfused with branched-chain ketoacids, 15 to 25% of the ketoacid that disappeared reappeared as the corresponding amino acid.

The lower peak level of BCAAT in the rabbit than in the rat, and the somewhat more uniform distribution along the nephron, is consistent with its herbivorous nature and the consequent lower need for urinary NH_3 . It is also significant that in the

rabbit, glutamine synthase is widespread along the nephron and is present in the thick ascending limb, although not at as high a level as in the proximal convoluted and straight segments [21].

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References

1. ROWSELL EV: Transaminations with L-glutamate and α -oxoglutarate in fresh extracts of animal tissues. *Biochem J* 64:235–245, 1956
2. ICHIHARA A, KOYAMA E: Transaminase of branched chain amino acids. I. Branched chain amino acid α -ketoglutarate transaminase. *J Biochem* 59:160–169, 1966
3. ICHIHARA A, NODA C, GOTO M: Transaminase of branched chain amino acids. High activity in pancreas and stomach. *Biochem Biophys Res Commun* 67:1313–1317, 1975
4. MILLER RH, HARPER AE: Branched-chain aminoacid metabolism in the isolated perfused rat kidney. *Fed Proc* 43:543, 1984
5. TISCHLER ME, GOLDBERG AL: Leucine degradation and release of glutamine and alanine by adipose tissue. *J Biol Chem* 255:8074–8081, 1980
6. TANAKA K, ROSENBERG LE: Disorders of branched chain amino acid and organic acid metabolism, in *The Metabolic Basis of Inherited Disease* (5th ed), edited by STANBURY JB, WYNGAARDEN JB, FREDRICKSON DS, GOLDSTEIN JL, BROWN MS, New York, McGraw Hill, 1983, pp 440–473
7. WESSON DE, MITCH WE, WILMORE DW: Nutritional considerations in the treatment of acute renal failure, in *Acute Renal Failure* edited by BRENNER BM, LAZARUS JM, Philadelphia, W.B. Saunders Co., 1983, pp 618–642
8. MITCH WE, COLLIER VU, WALSER M: Treatment of chronic renal failure with branched chain keto acids plus other essential amino acids or their nitrogen free analogues, in *Metabolism and Clinical Implications of Branched Chain Amino and Keto Acids*, edited by WALSER M, WILLIAMSON JR, Elsevier North Holland Inc., Amsterdam, 1981, pp 587–592
9. MITCH WE, CHAN W: Transamination of branched-chain keto acids by isolated perfused rat kidney. *Am J Physiol* 235:E47–E52, 1978
10. HAUSCHILD S, BRAND K: Comparative studies between ratio of incorporation of Branched-chain amino acids and their α -ketoanalogues into rat tissue proteins under different dietary conditions. *J Nutr Sci Vitaminol* 30:143–152, 1984
11. HINTZ C, TURK WR, CAMBON N, BURCH HB, LOWRY OH: A method for branched chain amino acid aminotransferase in microgram and nonogram tissue samples.
12. CHAN AWK, BURCH HB, ALVEY TR, LOWRY OH: A quantitative histochemical approach to renal transport. I. Aspartate and glutamate. *Am J Physiol* 229:1034–1044, 1975
13. LOWRY OH, PASSONNEAU JV: *A Flexible System of Enzymatic Analysis*, New York, Academic Press, 1972, pp 221–260
14. KAISLING B, KRIZ W: Structural analysis of the rabbit kidney, in *Advances in Anatomy, Embryology, and Cell Biology*, New York, Springer-Verlag, 1979, vol 56, pp 1–123
15. KATO T, BERGER SJ, CARTER JA, LOWRY OH: An enzymatic cycling method for nicotinamide adenine dinucleotide with malic and alcohol dehydrogenases. *Anal Biochem* 53:86–97, 1973
16. COHEN JJ, KAMM DE: Renal metabolism: Relation to renal function, in *The Kidney* (2nd ed), edited by BRENNER BM, RECTOR LC JR, Philadelphia, W.B. Saunders Co., 1981, pp 178–196
17. ROWSELL EV, TURNER KV: Subcellular distribution of α -ketoglutarate transaminases in rat kidney (abstract). *Biochem J* 89:64P, 1963
18. TAYLOR RT, JENKINS WT: Leucine aminotransferase. I. Colorimetric assays. *J Biol Chem* 241:4391–4395, 1966
19. ODESSEY R, GOLDBERG AL: Leucine degradation in cell-free extracts of skeletal muscle. *Biochem J* 178:475–489, 1979
20. BURCH HB, BROSS TE, BROOKS CA, COLE BR, LOWRY OH: The distribution of six enzymes of oxidative metabolism along the rat nephron. *J Histochem Cytochem* 32:731–736, 1984
21. CHAN AWK, PERRY SG, BURCH HB, FAGIOLI S, ALVEY TR, LOWRY OH: Distribution of two amino transferases and D-amino acid oxidase within the nephron of young and adult rats. *J Histochem Cytochem* 27:751–755, 1979
22. BURCH HB, CHOI S, MCCARTHY WZ, WONG PY, LOWRY OH: The location of glutamine synthase within the rat and rabbit nephron. *Biochem Biophys Res Commun* 82:498–505, 1978
23. GOOD DW, KNEPPER MA, BURG MB: Ammonia and bicarbonate transport by thick ascending limb of rat kidney. *Am J Physiol* 247:F35–F44, 1984